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PRINCIPAL INVESTIGATOR: Daniel V. Landers, M.D.

CONTRACTING ORGANIZATION: Magee Women's Hospital
Pittsburgh, Pennsylvania 15212-3180

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Cervical/vaginal and urinary tract infections occur commonly among 17-25 year old women and pose a significant problem for military women especially on deployment. This project is to develop a rapid "self-test kit" for common, treatable cervical/vaginal and urinary tract infections.

We made substantial progress in the development this kit combining lactoferrin dipstick to detect *Trichomonas vaginalis, Chlamydia trachomatis and Neisseria gonorrhoeae,* combined with a pH/amine test card to detect bacterial vaginosis and the leukocyte esterase/nitrite dipstick to detect urinary tract infection.

A number of problems were encountered including suboptimal specificity. Modifications are underway to address each of these problems and will be incorporated into the kit before testing by 300 women.

Despite the difficulties outlined above, the self-test kit results suggested appropriate treatment in the majority of cases. Specifically, 90% of women with BV, 84% of women with an STD and 87% of women with BV and/or an STD were targeted for appropriate therapy based on lactoferrin and pH/amine testing. Overall, among 99 women with self-test results, 63% would have received appropriate treatment. Planned improvements in the sensitivity/specificity of these tests will significantly enhance these results. Thus, we remain optimistic that we will develop a successful self-test kit.

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FOREWORD

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Daniel M Lander MD 10/14/97

PI - Signature Date

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Introduction

The primary goal of this proposal is to develop a "self-test kit" to be used by military women in the rapid diagnosis of the common, treatable cervical/vaginal and urinary tract infections. Testing will be performed on self-collected vaginal (introital) swabs (Q tips) and a urine sample. The secondary goal is to confirm the effectiveness of treating these infections with currently available, effective, single dose, low toxicity agents that could be included in a "self-care kit" (self-test kit plus single dose treatment packs) or administered by medical personnel in the field. The specific technical objectives of this proposal are:

1. To adapt the vaginal lactoferrin test to a simple, easily readable dipstick test to identify infection with *Trichomonas vaginalis*, *Chlamydia trachomatis* and/or *Neisseria gonorrhoeae*;

2. To evaluate vaginal amine and pH testing in a simple, easily readable test for the diagnosis of bacterial vaginosis and *Trichomonas vaginalis*;

3. To combine the vaginal lactoferrin, pH/amine test and urine leukocyte esterase/nitrite dipstick into a simple to use and easy to understand "self-test kit;"

4. To develop a simple and reliable algorithm for military women that combines symptomatology with rapid dipstick testing of vaginal fluid and urine which accurately predicts the presence of cervical/vaginal or urinary tract infections;

5. To test subjects' ability to select appropriate single dose treatment based on symptom/testing algorithm;

6. To demonstrate successful identification and eradication of infections predicted by "self-test kit," verified by "gold standard" diagnostic testing and treated with single dose, low toxicity antimicrobial agents.

Infections of the urogenital tract, particularly by sexually transmitted organisms, are a common and important health related problem to military women. These infections not only affect the mental and physical health of women, they may also adversely affect the ability of military women to perform their duties. These conditions and symptoms may also cause embarrassment to women working and living in close quarters. Additionally, these conditions lead to decreased productivity and time off from the workplace for evaluation, diagnosis, and treatment. All of these factors may significantly impact the ability and readiness of military women to perform their assigned tasks and duties. Furthermore, the adequately trained health care providers, laboratories, and advanced technology required for rapid diagnosis and treatment of these conditions may not always be readily available to deployed military women especially while in remote areas or developing countries. Speculum examination requiring special tables, stirrups, directed lighting, and other specialty equipment may not be easily accessible in many deployment situations.

Cervicitis, vaginitis, and urinary tract infections occur in upward of 20 million women each year in the United States.[1-4] These infections occur most commonly in the 2nd, 3rd, and 4th decade of life. The prevalence of these infections is highest in the 17-25 year old age group particularly the STDs.[2,5] Thus, these infections will commonly occur among women in the U.S. Armed Services by virtue of their age range alone. Recent reports from a survey of Army personnel indicate that 1 in 5 of women respondents reported having at least one STD over a 2 year period.[6] Deployed military men frequently engage in high-risk sexual behavior, and contract STDs. In one study, of almost 2000 military men deployed to South America, West Africa, and the Mediterranean, nearly half reported prior sexual contact with a commercial sex-worker and 1 in 5 reported a history of an STD before deployment.[7] High risk sexual behavior did not change. Over the next six-month deployment, almost half reported sexual contact with a commercial sex-worker, 1 in 10 acquired a new STD, and 1 in 10 military men reported inconsistent condom use.[7] Sexual exposure to men engaging in unsafe sexual practices increases the transmission of STDs among women. In a study

of nearly 500 active duty asymptomatic women reporting for routine annual exams, nearly 1 in 10 tested positive for *Chlamydia.*[8]

Recent preliminary reports from a survey of Army personnel indicated that 18% of women respondents report having had at least one STD over a 2 year period, and this may be an underestimate especially if women with an STD history were less likely to respond to the survey.[6] In another study of 476 asymptomatic active duty army women presenting for routine pap smears, 39 (8.2%) tested positive for chlamydia.[8] This is a high rate of asymptomatic chlamydia infection. These statistics are further compounded by the facts that only about 50% of all unmarried military personnel report using a condom during last intercourse and women under the age of 25, the age group at highest risk for acquiring an STD, account for two-thirds of the enlisted women that are pregnant at any given time.

There is additional accumulating evidence that other, less obvious, factors may influence the high rate of STDs among military women. Statistics show that 31% of women on active duty in the U.S. Army smoke cigarettes and 17% are heavy smokers.[6] This is significantly higher than the number of smokers in the general population.[6] Several recent studies have demonstrated that smoking is a significant risk factor in the acquisition of numerous STDs including *Chlamydia trachomatis, Neisseria gonorrhoeae*, and pelvic inflammatory disease and its sequelae.[9,10,11]

Delayed diagnosis and treatment of STDs and urinary tract infections may well lead to significant, even life threatening long-term sequelae. Serious renal infections, permanent infertility and life-threatening ectopic pregnancies are all recognized and well documented sequelae of lower urogenital tract infections in women.[1-4] Recent studies also indicate that the presence of these cervical/vaginal STDs significantly increase the risk of HIV acquisition.[12,13]

The most common forms of lower urogenital tract infections in women are cervical and vaginal infections (cervicitis and vaginitis) and bladder or urethral infections (cystitis or urethritis). The sexually transmitted organisms *Neisseria gonorrhoeae* and *Chlamydia trachomatis* are responsible for most cases of cervicitis while *Trichomonas vaginalis, Candida* species, and bacterial vaginosis account for nearly all cases of infectious vaginitis/vaginosis.[2-4,14,15]

Chlamydial infections are the most common bacterial STDs in the developed world. There are an estimated 4 million chlamydial infections annually in the United States alone with over 2 million occurring in women.[2,5,16] Over 2 million cases of gonorrhea occur in the United States each year.[2] Presenting complaints include vaginal discharge, dysuria, and abnormal uterine bleeding. Both gonorrhea and chlamydia can and often do present with minimal or very subtle symptoms necessitating screening and/or testing for minimal symptomatology in the "at risk" populations. Sequelae of these infections include pelvic inflammatory disease, ectopic pregnancy, permanent infertility and chronic, often debilitating pelvic pain.[2,5]

Infectious vaginitis and vaginosis account for some 8-10 million outpatient visits a year in the United States.[17] The three conditions accounting for the vast majority of these cases are trichomonas vaginitis, candida vaginitis, and bacterial vaginosis.

Vaginal yeast infections commonly occur in women. It has been estimated that 75% of women will have at least one episode of yeast vulvovaginitis, with 40-45% having two or more episodes.[18] The predominant organism causing these infections is *Candida albicans,* and occasionally non-albicans candidal species (*Candida tropicalis, Candida(Torulopsis) glabrata* or other Candida species). The most common presenting complaint is vaginal and/or vulvar pruritis with or without vaginal discharge, however, some 30% of women with yeast infections may present with discharge alone.[19]

An estimated 3 million cases of trichomoniasis occur in the United States annually. This infectious form of vaginitis is caused by *Trichomonas vaginalis*, a sexually transmitted motile protozoan.[15]

It accounts for approximately 10-15% of all cases of clinically evident vaginal infections. Infection with this organism is most often characterized by a copious, foul smelling discharge, but the clinical presentation can be quite variable including a significant number of women without specific vaginal complaints.

Bacterial vaginosis (formerly known as Gardnerella vaginitis, Haemophilis vaginitis, and nonspecific vaginitis) is the most common cause of malodorous vaginal discharge in women.[18] It has been estimated to be the etiology in as many as 45% of women with vaginitis/vaginosis.[15] Bacterial vaginosis (BV) is caused by a shift in the vaginal flora from the normal high concentrations of hydrogen peroxide-producing lactobacilli to a mixed flora consisting of high concentration of anaerobic organisms, *Gardnerella vaginalis*, and *Mycoplasma hominis*.[20] This shift in flora is associated with a homogenous, white vaginal discharge, elevated pH (>4.5), the production of amines, and the presence of clue cells.

Urinary tract infections, especially bladder infections (cystitis), are the most common bacterial infection in adult women accounting for over 7 million office visits per year in the United States.[2-4] Lower urinary tract infections may involve the urethra or the bladder. The usual presentation is internal dysuria (not external dysuria which is more associated with vulvar or vaginal infection). Acute urethritis is most often due to *Chlamydia trachomatis* or *Neisseria gonorrhoeae*. The vast majority of lower urinary tract infections in women are cystitis rather than urethritis. Acute, uncomplicated cystitis in young women is caused by *Escherichia coli* 80-90% of the time. The remaining 10-20% are caused by a variety of other organisms usually Gram negative bacteria including *Klebsiela, Proteus, Enterobacter, Pseudomonas* spp., and less commonly the Gram positive *Stapylococcus saprophyticus*, group B streptococci, and enterococci. Pyelonephritis generally a sequelae of cystitis, is recognizable by fever and lower back pain in addition to dysuria. This condition can require hospitalization and even lead to sepsis.

In summary, urogenital infections are common among military women as in the civilian population, but the nature of deployment may complicate the diagnosis and treatment of these infections. A rapid diagnostic test that could be self-administered in the field without the need for special medical facilities would be logistically and economically advantageous. Single dose treatments are now available and within the standard of care. The 1997 Centers for Disease Control guidelines for the Treatment of Sexually Transmitted Diseases (STDs) are due for release in the next few months and promise to include even more single dose treatments effective against the urogenital infections targeted by this proposal.

Body

The first year of this project was divided into two phases in the original proposal. Phase I, aimed primarily at development of the self-test kit and the data forms and Phase II, aimed at collecting specimens from 100 women and evaluation and refinement of the self-test kit. Each "Statement of Work" task listed in the original proposal is printed in italics and addressed separately below.

The nature of this study required that Phase I and II be carried out simultaneously to optimize the available time in accomplishing our stated goals. The overall goal of Phase I and II was to develop our proposed self-test kit, and to compare its sensitivity and specificity to gold standard testing. The intent is to develop a self-test kit that will indicate curative treatment regimens. The primary goal of Phase II was to assess and modify the self-test kit to optimize diagnostic accuracy and treatment efficacy before recruiting 900 more women to test the kit (Phase III).

As outlined in the "Statement of Work" in our original proposal and in accordance with the specific objectives of this project we began work on 6 tasks in Phase I and 6 tasks in Phase II of this project.

Phase I Tasks:

1. *Determine optimal test format for the Lactoferrin dipstick including establishment of cutoff and appropriate threshold for sensitivity.*

   - Lactoferrin dipsticks were provided by TechLab, Blacksburg, VA, and have highly correlated with enzyme-linked immunosorbant assay (ELISA) values. We performed spike and recovery with serial dilutions and found a 95% correlation with ELISA levels ≥500 ng/ml. We have now studied 100 samples and correlated dipstick results with levels determined by ELISA.

   - We have also tested vaginal swabs for lactoferrin by ELISA and dipstick on 98 of the first 100 women recruited as a part of Phase II (see below) and analyzed this data for sensitivity and specificity in predicting the presence of one or more of the three targeted STDs (*Trichomonas vaginalis, Chlamydia trachomatis*, and *Neisseria gonorrhoeae*). These results are described below under Phase II, task 2. Serial dilutions were also done on all 98 samples and the ELISA results were comparable to the dipstick results. The correlation remained above 95% through all dilutions.

2. *Combine the vaginal lactoferrin, pH/amine test, and urine leukocyte esterase/nitrite dipstick into a simple to use and easy to understand self test kit.*

   - The lactoferrin dipstick is able to detect levels above 500 ng/ml with a high degree of accuracy and will be included in the self-test kit. It is possible that a defensin dipstick will also be developed that may be included in the kit to enhance the specificity of lactoferrin in predicting the presence of the targeted STDs. The reasoning for this is detailed below under Phase II, task 2.

   - The pH/Amine test (FemExam card from Litmus Concepts, Calif, USA) is now FDA approved for the diagnosis of bacterial vaginosis and will be included in the test kit. New cards have been produced to rectify a quality control issue discovered during Phase II testing. (See below under Phase II, task 2.)

   - We are currently collaborating with Litmus Concepts in the development of a yeast detection card able to identify the presence of Candida species which would significantly enhance the sensitivity and specificity of our test kit. Preliminary in vitro results on this

new rapid test card are encouraging and a pilot card will be available during the second year of this project.

- The leukocyte-esterase dipstick has been commercially available for some time now from Bayer Corporation (Elkhart, IN). We are now comparing several different leukocyte-esterase dipsticks (companies) to determine which has the best sensitivity and specificity for inclusion in the self-test kit (see Phase II, Task 2).

3. *Prepare IRB application and create patient consent forms for IRB approval and patient enrollment.*

   - The IRB application has been prepared and approved at our institution and has been reviewed by The Surgeon General's Human Subject Research Review Board and has been approved contingent on revisions, which have been made and submitted.

4. *Establish data collection instruments for patient demographics and relevant specimen information.*

   - Detailed data collection instruments have been created and tested in the first 100 patients. These forms are included in the appendix.

5. *Develop a database for this information.*

   - An extensive database has been developed and is currently being used in our evaluations. The database contains 349 variables. The variables include information on demographic and behavioral characteristics, symptoms, results of physical examination, and laboratory testing. The data are written onto scannable forms, scanned, verified, labeled and coded, and imported to statistical package (SPSS for Windows) for descriptive analysis.

6. *Develop patient instruction sheets for sample collection and performance of the rapid test kit.*

   - Patient instruction sheets have been created for the collection of vaginal swabs and are included in the appendix. These sheets have been tested in over 300 patients collecting vaginal swabs for Chlamydia PCR testing. These sheets have assisted women in self-collecting specimens that yielded results similar to those obtained on simultaneous clinician-collected samples.

Phase II Tasks:

1. *Begin recruitment and patient sampling for the self-test kit development phase.*

   - Women presenting to the study sites with complaints of dysuria or vaginal discharge, itching, burning or irritation, between the ages of 18-40 were recruited as study participants. The exclusion criteria for the study were the use of antibiotics or other treatment for urogenital infections in the past two weeks and age outside the specified age range. During the clinic visit a complete medical history was taken. Upon completion, a pelvic exam was performed on each woman. The clinician collected three simultaneous vaginal (introital) swabs and performed the pH/amine test card, the lactoferrin dipstick, the leukocyte-nitrite dipstick, a wet mount for microscopic examination and recorded the results of each. A clean, unlubricated speculum was placed into the vagina, and 6 sterile dacron swabs were used to obtain vaginal material from the posterior vaginal fornix and from the endocervix. The following tests were performed to evaluate the self-test results and to determine the exact infectious agents present: Swab #1: Lactoferrin/Defensins

ELISA, Swab #2: PCR for *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and *Trichomonas vaginalis*, Swab #3: bacterial vaginosis by gram stain, Swab #4: *Trichomonas* culture, Swab #5: Yeast culture, and Swab #6: *N. gonorrhoeae* culture (cervical).

- Recruitment and patient sampling was achieved ahead of schedule resulting in the enrollment of 100 women. The demographics of these women are displayed in Table 1.

Table 1. Demographics of Women Enrolled in Phase I

Age		Race		Marital Status	
Mean = 25.9 yrs.		African-American	55%	Single	77%
S.D. = 5.9 yrs.		European-American	39%	Married/Cohabitating	12%
		Multiethnic	4%	Separated/Divorced	8%
		Other	2%		
Years Education		**Employment Status**		**Tobacco Use**	
Mean	13.25 yrs.	Employed	66%	Any Smoking	60%
S.D.	2.63 yrs.	Full-Time	41%	Heavy Smokers:	
		Part-Time	25%	≥1ppd	13%
		Unemployed (includes students)	34%	Non-Smokers	40%
Alcohol Use		**Douching Habits**			
Any Use	66%	Ever	72%		
Heavy Use		Never	28%		
Daily	5%				
None	34%				

All women recruited had at least one urogenital complaint including vaginal discharge in 73%, pruritis in 49%, abnormal vaginal odor in 47%, and burning or pain in 35%. The "gold standard" testing for urogenital tract infections are shown in Table 2.

Table 2. Gold Standard Testing

Infection	Pathogen	"Gold Standard Test"
Cervicitis/Urethritis	*C. trachomatis* / *N. gonorrhoeae*	PCR and Culture / Culture
Bacterial vaginosis	Multiple	Gram stain
Candida vaginitis	*Candida*	Culture
Trichomoniasis	*T. vaginalis*	PCR and Culture
Urinary tract	Coliforms	Culture

Among the first 100 patients, gold standard testing revealed that 41 women had bacterial vaginosis (BV), of which, 11 women had a concurrent sexually transmitted disease (STD: N. *gonorrhoeae*, *C. trachomatis*, or *T. vaginalis*) and 30 women had BV alone. There were an additional 14 women with one of the STDs that did not have BV. Overall, 26 women had one or more of the STDs, specifically 15 had *T. vaginalis,* 9 had *C. trachomatis,* and 4

had *N. gonorrhoeae*. Yeast was cultured from 17 women and in the remaining 27 women, no cervical/vaginal pathogens were identified. These results are similar to our overall population of women with genital complaints. Visual and bimanual examinations did not reveal evidence of any other vaginal disease such as genital herpes or human papilloma virus that might account for vaginal complaints in women without pathogens.

2. *Analyze test kit performance compared with "gold standard" test results and evaluate the kit's accuracy in predicting the presence of cervical/vaginal or urinary tract infections.*

- Lactoferrin Testing for STDs:

Lactoferrin levels were determined using the Leuko-ELISA Kit (TechLab, Blacksburg, VA). The vaginal sample is diluted 1:20 in kit diluent and a 100ul aliquot is added to an antibody coated 96 well microtiter plate. The plates are incubated at 37°C for 10 min., washed, conjugate is added and the plate is incubated at 37°C for 10 min. The wash step is repeated and 1 drop of substrate is added, the plate is incubated at room temperature for 5 minutes. Following the substrate incubation, 1 drop of color intensifier is added and the plate is read at 450nm. Standard curves were generated using purified human lactoferrin. The detection limit on the assay is 4ng/ml. Values above the upper limit of the assay were rediluted and repeated. The lactoferrin ELISA was performed in Dr. Phillip Heine's laboratory, by his Laboratory Supervisor, Leo Mortimer. The data was analyzed by Dr. Heine.

Lactoferrin levels were determined on clinician-collected vaginal swabs obtained from 100 women. Results were obtained from 98 samples (2 specimens were contaminated during assay preparation). The data on these women was analyzed using two different cutoff values to optimize sensitivity and specificity. The first cutoff value used was 500 ng/ml corresponding to the dipstick detection value. The second cutoff value used was 200 ng/ml. The sensitivity/specificity calculations are shown in Table 3.

Table 3. Sensitivity, specificity, positive predictive value, and negative predictive value at a 200 ng/ml cutoff point and a 500 ng/ml cutoff point.

	STD (TV,CT, or GC) (+)	STD (TV,CT, or GC) (-)	
Lactoferrin ≥ 200	23	44	67
Lactoferrin < 200	3	28	31
	26	72	98

Sensitivity = 89%
Specificity = 39%
PPV = 34%
NPV = 90%

	STD (TV,CT, or GC) (+)	STD (TV,CT, or GC) (-)	
Lactoferrin ≥ 500	21	36	57
Lactoferrin < 500	5	36	41
	26	72	98

Sensitivity = 81%
Specificity = 50%
PPV = 37%
NPV = 88%

The fact that nearly 90% of women with an STD had a positive lactoferrin test (sensitivity = 89%) was encouraging. In symptomatic women, where the prevalence is reasonably high, the negative predictive value is also high (90%).

The specificity however was only 39% and the positive predictive value only 34%. As we analyzed the data it was clear that a significant number (12 of 17) of women with yeast vaginitis had a positive lactoferrin test. This could be problematic since we do not have a rapid test for yeast and our yeast diagnosis is based on symptoms of pruritis. Fortunately, we will be able to improve this aspect of the test with the development of a yeast card (see below).

We have also begun to evaluate other soluble White Blood Cell (WBC) products that can be measured in vaginal fluid and are potential candidates for colorimetric card or dipstick testing. Defensins are human neutrophil peptides found in quantities as high as 5 pg/cell. This is the most abundant neutrophil protein, and is stable to prolonged storage and proteolysis. These characteristics make this protein an ideal candidate for the research setting. Defensins are used as markers for sepsis and meningitis.[21,22] We have begun ELISA testing for defensins. The mean defensin level measured from vaginal swabs in 26 women with an STD in our Phase I/II patients was 17,682 ng/ml compared with the 8,899 ng/ml mean value among 27 women without identifiable pathogens by "gold standard" tests. In our initial attempts at determining a cutpoint for defensins, we looked at levels ≥ 5,000 ng/ml as being significant (positive). When we combined defensin values with lactoferrin we achieved a sensitivity of 77% and a specificity of 65% (Table 4). If women with yeast are not included (i.e. could be identified by a yeast card), the sensitivity remained 77% and the specificity rose to 75%. These sensitivity/specificity values exceed those attained by standard testing currently used in clinical settings across the United States today. These standard tests include wet mount for the diagnosis of *T. vaginalis*, cultures for *N. gonorrhoeae* and chlamydiazyme for *C. trachomitis*. In this group of 26 women with STDs, 10/15 patients with *T. vaginalis* infection and 7/9 *with C. trachomitis* were identified by these standard tests. Thus, the lactoferrin/defensin test was more accurate than standard tests in identifying the presence of one of the three tested STDs.

Table 4. Sensitivity and Specificity of lactoferrin and defensin results of all patients with or without an STD.

All Participants

Lactoferrin 200	Defensin 5000	STD (+)	STD (-)
(+)	(+)	20	38
(+)	(-)	3	5
(-)	(+)	0	16
(-)	(-)	3	12
		26	71

Sensitivity = 77%
Specificity = 65%
PPV = 44%
NPV = 88%

All Participants Excluding Yeast

Lactoferrin 200	Defensin 5000	STD (+)	STD (-)
(+)	(+)	20	14
(+)	(-)	3	18
(-)	(+)	0	6
(-)	(-)	3	17
		26	55

Sensitivity = 77%
Specificity = 75%
PPV = 59%
NPV = 87%

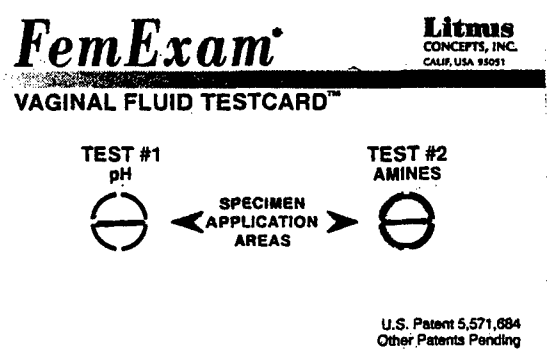
Another important factor relevant to cure rates following single dose treatment is the overlap in treating BV and TV. Among our first 100 patients, 26 had one or more of the STDs, the most prevalent of which was TV (15/26). Concurrent BV was identified in 11 women with an STD, and our treatment algorithm (Table 5) for BV is metronidazole 2g, which is also the treatment for TV. Thus, in many cases, women with TV would be cured because of their BV treatment even when their self-test doesn't identify TV. This may further enhance the ability of self-test results to guide selection of curative single dose treatment. This is discussed further below in the section on cure rates.

- pH/Amine Testing for BV:

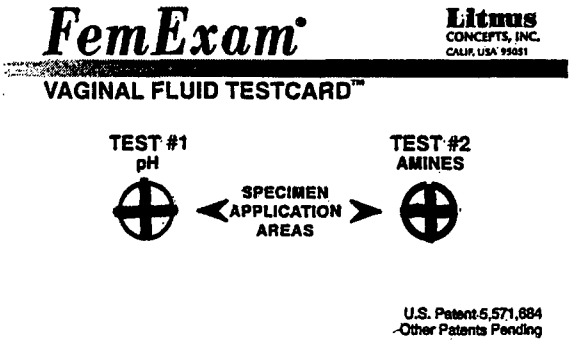
The rapid diagnosis of BV is based on pH and volatile amines (trimethylamines) using the FDA-approved (as of February 1997) FemExam card (Litmus Concepts, Calif, USA)(Figure 1).

Figure 1. Example of a Negative and Positive FemExam Card

Negative Result



Positive Result



In collaboration with Dr. Paul Lawrence at Litmus Concepts we obtained 100 test cards from a single production lot. Our ongoing quality assurance program led to a review of the first 54 tests which indicated a sensitivity of 91% but a specificity of only 46%. This was of particular concern to us since we were involved along with four other centers in testing of this card in over 600 women prior to FDA approval which showed this device to be highly specific as well as sensitive in the diagnosis of BV. We changed to using cards from a separate lot for further testing and Dr. Lawrence and colleagues at Litmus Concepts began an investigation into the possible cause of decreased specificity. Meanwhile we tested an additional 45 patients (one specimen was mishandled) with cards from a separate lot and although specificity improved to 65%, this was well below the demonstrated capability of the card. After intense investigation at Litmus Concepts it was discovered that a whole block of cards had been produced with a barrier layer thinner than the original acceptable limits, leading to an excess of secretions being allowed to contact the test envelope. This caused an inordinate number of false positive tests that would explain our lack of specificity. The overall sensitivity and specificity in the first 99 patients was 85% and 54% respectively, despite the manufacturing defect. The quality assurance program at Litmus Concepts is now able to detect any variations in barrier thickness and should prevent further variations in sensitivity and specificity. We plan to test an additional 50 women before finalizing our self-test kits.

As we noted above, the single dose treatment for BV and TV are the same. Women with either TV or BV will receive curative therapy if either the lactoferrin or pH/amine tests are positive. Among the 40 evaluable women with BV (one pH/amine test specimen was mishandled as noted above), 35 (87.5%) would have received curative therapy based on self-test kit results.

- Yeast Diagnosis by Algorithm:

Our original algorithm (shown in Table 5) depended on women having symptoms of pruritis or burning in the absence of positive lactoferrin and pH/amine testing.

Table 5. Proposed Treatment Algorithm

pH/Amine	Lactoferrin	Leukocyte Esterase/Nitrite	Proposed Treatment
+	+	+	Azithromycin, Ciprofloxacin Metronidazole & Fleroxacin
+	+	-	Azithromycin, Ciprofloxacin & Metronidazole
+	-	-	Metronidazole
+	-	+	Metronidazole & Fleroxacin
-	+	+	Azithromycin, Ciprofloxacin & Fleroxacin
-	-	+	Fleroxacin
-	-	-	If pruritis is present treat with Fluconazole

There were 24 women culture positive for Candida of which 17 were negative by all other gold standard testing. The algorithm would have directed only one to be treated because 9 women with yeast had a positive pH/amine test and the remainder had a positive lactoferrin test. Since a total of 60 women had pruritis or burning it was also not reasonable to treat women based on pruritis regardless of lactoferrin or pH/amine testing. While this was discouraging to see, there is reason to be optimistic in overcoming this setback. First, we anticipate dramatically reducing the number of false positive Litmus Card tests as described above. This accounted for 9 women not being treated with antifungal therapy. Second, the specificity of our STD detection may well be enhanced by adding defensin testing. Finally our collaborator, Dr. Paul Lawrence at Litmus Concepts, has already made significant progress towards developing a Yeast Card to detect vaginal candida and we anticipate evaluating that test clinically in the very near future.

- Urinary Tract Infections by Leukocyte /Esterase Testing:

Urinary tract infections were detected by culture in 16/100 (16%) of women. Only 6 of these were symptomatic with dysuria. Leukocyte/esterase dipsticks identified only one of the 6 women with positive cultures. The majority of positive urine cultures were in women without dysuria. We are also testing our stored samples with several other commercially available leukocyte/esterase dipsticks to find enhanced sensitivity. The claimed sensitivity of commercially available dipsticks is well above 90%.

- Women Without Identifiable Pathogens:

There were 27/100 women presenting with urogenital tract symptoms that were found to have all negative testing by "gold standard" tests. This is not unexpected and is consistent with our outpatient clinics and many other populations of symptomatic women. In the typical clinical setting these women are treated empirically, based on symptoms until the results of cultures or other type of diagnostic testing become available. Our rapid tests selected for the self-test kit

correctly ruled out infection in 14 (52%) of these women obviating the need for unnecessary antimicrobial therapy.

- Overall cure rates:

To determine the effectiveness of a self-test kit aimed at directed single dose therapy, the cure rates will be dependent on the number of women directed toward effective therapy for the pathogens they harbor. It must be remembered that in the United States, the best medical centers currently use wet mount for the diagnosis of TV, yeast and bacterial vaginosis, clinical diagnosis with empiric therapy backed-up by chlamydiazyme and culture for CT and NG, respectively, and urine microscopy with culture back-up for urinary tract infections. The sensitivity and specificity of wet mount readings is notoriously poor. Empiric diagnosis of CT and NG is even worse and dependent on the prevalence in the population.

In this study our first 98 evaluable patients were tested with lactoferrin dipstick, pH/amine testing and leukocyte-esterase determination. They would have been assigned therapy based on the treatment algorithm (Table 5) if on deployment. Based on this testing, 90% of the women with BV alone, 84% of women with an STD and 87% of women with BV and/or an STD would have received efficacious therapy. If we include those women with yeast vaginitis and those without pathogens, 63% of women would have received efficacious therapy. If a yeast diagnostic card or test would have been available that number would rise to 87%. If our defensins test improves the sensitivity/specificity of the lactoferrin test as our preliminary data indicates (see above) and our replacement pH/amine test cards are more specific (see above) then our self-test kit may well direct efficacious therapy in well over 90% of women which would far exceed the current rate in clinics across the United States today.

3. *Analysis of patients' ability to select appropriate single dose treatment based on symptom/testing algorithm.*

- This task awaits completion of test kit modifications as described above.

4. *Make any and all modifications to the test kit based on findings from the developmental phase data and make a final form of the kit.*

- Many modifications to the kit are underway as described above in Phase II, task 2. These modifications include: 1) Re-analysis of the lactoferrin data to determine if there is a more specific cutpoint for dipstick testing; 2) Performing ELISA testing for vaginal defensins, determine a cutoff and combine data with lactoferrin data to improve the sensitivity and specificity of detecting the presence or absence of an STD; and 3) Repeat testing on 50 women including the use of the FemExam card with the appropriate barrier layer thickness. In addition, we will be testing the vaginal yeast card under development by Litmus Concepts in the coming year. We will also be testing additional, commercially available leukocyte/esterase dipsticks.

5. *Refine and finalize instruction sheets as needed to improve the efficiency and scope of the data collection process.*

- Interview forms and instructions are reviewed on an ongoing basis and modifications will be made as is deemed appropriate. As patients begin to use self-test kit and interpret results this will become a very important task.

6. *Revise and finalize data collection sheets as needed to improve the efficiency and scope of the data collection process.*

- The data collection sheets (see appendix) are also reviewed on an ongoing basis to insure the validity and accuracy of collected and entered data. We have to date, entered complete data on 100 women enrolled during Phase II of this project.

Conclusions

The first year of this project was completed with significant progress being made in developing a rapid self-test for symptomatic cervical/vaginal and urinary tract infections in women. A number of problems were encountered as described above, including sub-optimal specificity of the lactoferrin and pH/amine tests. Troubleshooting and modifications have been or are being made to address each of these problems. We are planning to test an additional 50 women prior to finalizing our kit for Phase III. We have had no problem recruiting patients for this study and therefore anticipate remaining on schedule for completion of Phase III despite additional time being spent optimizing our self-test kits.

It is notable that despite the difficulties outlined above, the self-test kit results would have directed women to appropriate treatment in the majority of cases. Specifically, 90% of women with BV alone, 84% of women with an STD, and 87% of women with BV and/or an STD would have been directed to appropriate therapy based on lactoferrin and pH/amine testing. Planned improvements in the sensitivity/specificity of these tests will significantly enhance these results. Overall, including all 99 women with self test results, 63% of women would have received the appropriate treatment decision. If 90% of women with yeast had been identifiable using a yeast card test, then 87% of women with disease by gold standard testing would have been directed to take appropriate therapy. This number may well exceed the number treated appropriately in fully equipped clinical settings. Thus, we remain optimistic that a successful self-test kit can be developed for women with symptomatic urogenital infections.

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DOD STUDY
INTERVIEW FORM A

DODA
12/11/96

STUDY IDENTIFICATION

Study Code ☐☐☐☐

Interview Date ☐☐ / ☐☐ / ☐☐

Interviewer Code ☐☐

Enrollment Site

0 0. Pitt 0 1. Health Dept. 0 2. Other _____

DEMOGRAPHICS

1. How old are you? ☐☐

2. What is your date of birth? ☐☐ / ☐☐ / ☐☐

3. Describe your race / ethnicity?

○ 0. African American
○ 1. European American
○ 2. Hispanic
○ 3. Asian
○ 4. Native American
○ 5. Multi-ethnicity/bi-racial
○ 6. Other

4. What is your marital status?

○ 0. Single [live with partner, check below]
○ 1. Living with partner > or = 4 months
○ 2. Married
○ 3. Separated
○ 4. Divorced
○ 5. Widowed

5. Number of years you have been in school? ☐☐

6.* Are you *now either going to* school or on vacation from school?

○ 0. Yes
○ 1. No [skip to 8]

7. Kind of school you are going to?

○ 0. High school
○ 1. College
○ 2. Trade/vocational
○ 3. GED
○ 4. Other:_____
○ 5. N/A

8. Do you have a job?

○ 0. Unemployed
○ 1. Employed part-time
○ 2. Employed full-time

9. Have you ever had any major medical problems?

○ 0. No
○ 1. Yes; what problems?

Medical Code ☐☐ ☐☐ ☐☐

10. Had any antibiotics in the past 30 days?

○ 0. None
○ 1. Don't know
○ 2. Yes –>

How many days ? ☐☐

11. What antibiotic have you taken? ☐☐ ☐☐ ☐☐

12. How many days since the last dose? ☐☐

CIGARETTE, ALCOHOL & MARIJUANA USE

13.* During the **past 30 days**, how many cigarettes have you smoked? ☐☐☐☐

14.* During the **past 30 days**, on how many days did you have at least one drink of alcohol? ☐☐

15.* During the **past 30 days**, how many times did you use marijuana? ☐☐

DOD STUDY
INTERVIEW FORM A
STUDY CODE _____

GYNECOLOGICAL HISTORY

"Now we're going to ask some gyne questions"

16. Number of days since beginning of last period? [ ][ ][ ][ ]

17. Do you use pads, tampons, or both during your period?

- ○ 0. Pads [ask 18 then skip to 20]
- ○ 1. Tampons
- ○ 2. Both
- ○ 3. N/A

18. Do you currently use **deodorized** pads, tampons, or both, during your period?

○ 0. No ○ 1. Pads ○ 2. Tampons ○ 3. Both

19. During your last period, what brand of tampon did you use?

- ○ 0. Tampax
- ○ 1. Kotex
- ○ 2. Ob
- ○ 3. Playtex
- ○ 4. Store generic
- ○ 5. Other:_____

[ ][ ] Tampon Code

20. Have you ever douched?

- ○ 0. No [skip to 28]
- ○ 1. Yes

21. At what age did you first douche? [ ][ ]

22. On average, how many times do you douche each month? [ ][ ]

23. How long ago did you last douche? [days] [ ][ ][ ][ ][ ]

24. In the past 30 days, how many times did you douche? [ ][ ]

25. When you douche, what preparation do you use?

- ○ 0. N/A
- ○ 1. Water only
- ○ 2. Vinegar & Water
- ○ 3. Water & Baking Soda
- ○ 4. Chemical
- ○ 5. Other: _____

26. Why do you douche?

- ○ 0. N/A
- ○ 1. Post menses
- ○ 2. Post intercourse
- ○ 3. Vaginal D/C
- ○ 4. Vaginal odor
- ○ 5. Cleanliness
- ○ 6. "it's normal to do"
- ○ 7. Other: _____

27. Where did you first get the idea to douche? [who suggested or what motivated you to douche]

- ○ 0. N/A
- ○ 1. Mother
- ○ 2. Other female relative
- ○ 3. Partner
- ○ 4. Friends
- ○ 5. Commercials
- ○ 6. Other

28. In the past 4 months have you used any other vaginal over- the-counter products (including spermicides or sprays)?

- ○ 0. No [skip to 30]
- ○ 1. Yes

DOD STUDY
INTERVIEW FORM A
STUDY CODE _____

29.

What products did you use?	How long ago did you last use any of these products? [days]	On the average, how many times do you use these products each month?
Foam, Jelly or Cream ○ 0. Yes ○ 1. No ○ 2. N/A		
Film/supp ○ 0. Yes ○ 1. No ○ 2. N/A		
Lubricant ○ 0. Yes ○ 1. No ○ 2. N/A		
Douche ○ 0. Yes ○ 1. No ○ 2. N/A		
Deodorant ○ 0. Yes ○ 1. No ○ 2. N/A		
Yeast medication ○ 0. Yes ○ 1. No ○ 2. N/A		
Other medication ○ 0. Yes ○ 1. No ○ 2. N/A		
Lubricated condoms ○ 0. Yes ○ 1. No ○ 2. N/A		
Condoms with Non-oxynol 9 ○ 0. Yes ○ 1. No ○ 2. N/A		
Other: _____ ○ 0. Yes ○ 1. No ○ 2. N/A		

30. How long ago did you have your last pap smear? [months] ☐☐☐

31. Have you ever had an abnormal pap?

○ 0. No [skip the remainder of Form A questions]
○ 1. Yes
○ 2. Don't know [skip remainder of Form A questions]
○ 3. N/A

32. How long ago was it? [months] ☐☐☐

33. What was the result of your abnormal pap?

○ 0. Reactive, infection, benign atypical
○ 1. Mild dysplasia, CIN 1
○ 2. Moderate dysplasia, CIN 2
○ 3. Severe dysplasia, CIN 3
○ 4. Don't know
○ 5. N/A

Draft

DOD STUDY
INTERVIEW FORM B

STUDY IDENTIFICATION

Study Code ☐☐☐☐

Interviewer Code ☐☐

Interview Date ☐☐ / ☐☐ / ☐☐

Enrollment Site
0 0. Pitt
0 1. Health Dept.
0 2. Other _____

PREGNANCY AND BIRTH CONTROL

1.* How many times have you been pregnant? ☐☐ [if 0, skip to 4]

2. Are you currently pregnant? ○ 0. Yes ○ 1. No ○ 2. Not Sure

3. **For each pregnancy ask the following questions and complete in chronological order.**
What was the outcome?/ How many weeks were you pregnant?/ What year was it when it ended?

Pregnancy #	Outcome	Weeks	Year Ended
1	☐	☐☐	☐☐
2	☐	☐☐	☐☐
3	☐	☐☐	☐☐
4	☐	☐☐	☐☐
5	☐	☐☐	☐☐

Outcome Codes

1. Live birth [survived past 28 days]
2. Termination
3. Miscarriage
4. Stillbirth [>= 20 weeks gestational age]
5. Neonatal Death [live birth, death < 28 days]
6. Ectopic
7. N/A

4. Have you ever used any method of birth control? ○ 0. Yes ○ 1. No

5. What birth control methods are you using now?

None (0)	○ 0. Yes	○ 1. No	○ 2. N/A
Pills (1)	○ 0. Yes	○ 1. No	○ 2. N/A
Condoms only (2)	○ 0. Yes	○ 1. No	○ 2. N/A
Condoms + spermicide (3)	○ 0. Yes	○ 1. No	○ 2. N/A
Condoms + other methods (4)	○ 0. Yes	○ 1. No	○ 2. N/A
Spermicide only (5)	○ 0. Yes	○ 1. No	○ 2. N/A
Depo (6)	○ 0. Yes	○ 1. No	○ 2. N/A
Norplant (7)	○ 0. Yes	○ 1. No	○ 2. N/A
Abstinence (8)	○ 0. Yes	○ 1. No	○ 2. N/A
"Morning-after" pill (9)	○ 0. Yes	○ 1. No	○ 2. N/A
Diaphragm (10)	○ 0. Yes	○ 1. No	○ 2. N/A
Cervical cap (11)	○ 0. Yes	○ 1. No	○ 2. N/A
IUD (12)	○ 0. Yes	○ 1. No	○ 2. N/A
Withdrawal (13)	○ 0. Yes	○ 1. No	○ 2. N/A
Rhythm (14)	○ 0. Yes	○ 1. No	○ 2. N/A
Douching (15)	○ 0. Yes	○ 1. No	○ 2. N/A
Tubal Ligation (16)	○ 0. Yes	○ 1. No	○ 2. N/A
Virgin (17)	○ 0. Yes	○ 1. No	○ 2. N/A
Other (18) :_____	○ 0. Yes	○ 1. No	○ 2. N/A

6. How long have you been using this method of birth control? [months]

1 ☐☐☐
2 ☐☐☐
3 ☐☐☐
4 ☐☐☐

7. What method did you use before this? [Numerical code from list in question 5]

☐☐

8. How long did you use that method of birth control? [months]

☐☐

DOD STUDY
INTERVIEW FORM B
STUDY CODE _____

9. What birth control methods have you ever used in the past?

Pills (1)	○ 0. Yes	○ 1. No	○ 2. N/A
Condoms (2)	○ 0. Yes	○ 1. No	○ 2. N/A
elly/Foam/Film Only (3)	○ 0. Yes	○ 1. No	○ 2. N/A
Depo (4)	○ 0. Yes	○ 1. No	○ 2. N/A
Norplant (5)	○ 0. Yes	○ 1. No	○ 2. N/A
"Morning-after" Pill (6)	○ 0. Yes	○ 1. No	○ 2. N/A
Diaphragm (7)	○ 0. Yes	○ 1. No	○ 2. N/A
Cervical Cap (8)	○ 0. Yes	○ 1. No	○ 2. N/A
IUD (9)	○ 0. Yes	○ 1. No	○ 2. N/A
Withdrawal Only(10)	○ 0. Yes	○ 1. No	○ 2. N/A
Tubal Ligation (11)	○ 0. Yes	○ 1. No	○ 2. N/A
Virgin (12)	○ 0. Yes	○ 1. No	○ 2. N/A

10.* Have you ever been told by a doctor or nurse that you had any of the following infections?

○ 0. No ○ 1. Yes ○ 2. Don't know

11. If yes, how many times?
How old were you the most recent time you had that infection?

	# of times	Age most recent
Urinary Tract Infection (1)		
Pyelonephritis (2)		
Yeast (3)		
Bacterial Vaginosis (4)		
Trichomoniasis (5)		
Cervicitis (6)		
Pelvic Inflammatory Disease (7)		
Chlamydia (8)		
Gonorrhea (9)		
Syphilis (10)		
Human Papilloma Virus (11)		
Oral Herpes (12)		
Genital Herpes (13)		
Other: _____ (14)		

DOD STUDY
INTERVIEW FORM B
STUDY CODE _____

12. During the **past 30 days**, how many times did you have sex? [ ][ ][ ]

13.* During the **past 3 months**, with how many people did you have sexual intercourse? [ ][ ][ ]

14.* During your life, with how many people have you had sexual intercourse? [ ][ ][ ][ ]

15.* During this past year, with how many people have you had sexual intercourse? [ ][ ][ ][ ]

16. When was the last time you had sex? [days] [ ][ ][ ][ ]

17. Have you ever had sex during your period?

○ 0. No

○ 1. Yes

18. Have you ever had sex with an uncircumcised partner?

○ 0. No ○ 1. Yes ○ 2. Don't know

19. Have you ever had sex with a woman?

○ 0. No

○ 1. Yes

20. Have you had sex with a woman in the last week?

○ 0. No

○ 1. Yes

21. Have you ever had vaginal sex after having anal sex?

○ 0. No

○ 1. Yes

22. When this happens, how frequently does your partner wash in between?

○ 0. Always
○ 1. Most of the time
○ 2. Sometimes
○ 3. Rarely
○ 4. Never
○ 5. N/A

23. When this happens, how frequently does your partner use a new condom?

○ 0. Always
○ 1. Most of the time
○ 2. Sometimes
○ 3. Rarely
○ 4. Never
○ 5. N/A

24. Has anyone performed oral sex on you in the last week?
[i.e., has anyone gone down on you last week]

○ 0. No ○ 1. Yes

25. Have you performed oral sex on anyone in the last week?
[i.e., have you gone down on anyone last week]

○ 0. No ○ 1. Yes

DOD STUDY
INTERVIEW FORM B
STUDY CODE _____

Have you had any ot the following symptoms in the past week?

Vaginal Symptoms

26. Abnormal discharge in the past week
- ○ 1. Yes
- ○ 2. No
- ○ 3. Missing

27. Abnormal odor
- ○ 1. Yes
- ○ 2. No
- ○ 3. Missing

28. Discharge amount
- ○ 1. Less than normal
- ○ 2. Normal
- ○ 3. More than normal
- ○ 4. Missing

29. Discharge consistency
- ○ 1. Thicker than normal
- ○ 2. No change
- ○ 3. Thin / watery
- ○ 4. Missing

30. Discharge color
- ○ 1. White
- ○ 2. Clear
- ○ 3. Yellow
- ○ 4. Brown
- ○ 5. Bloody
- ○ 6. Missing

31. Pruritus
- ○ 1. Yes
- ○ 2. No
- ○ 3. Missing

32. Burning or pain
- ○ 1. Yes
- ○ 2. No
- ○ 3. Missing

Urethral Symptoms

33. Dysuria in the past week
- ○ 1. Yes
- ○ 2. No
- ○ 3. Missing

Abdominal Symptoms

34. Pain in the past week
- ○ 1. Yes
- ○ 2. No
- ○ 3. Missing

Draft

DOD STUDY
PHYSICAL EXAMINATION FORM

STUDY IDENTIFICATION

Study code ☐☐☐☐

Interviewer Code ☐☐

Interview Date ☐☐ / ☐☐ / ☐☐

LMP ☐☐ / ☐☐ / ☐☐

Weight ☐☐☐

Enrollment Site

0 0. Pitt 0 1. Health Dept. 0 2. Other _____

Abdominal Assessment

1. Abdomen assessible
   - ○ 1. Yes
   - ○ 2. No
   - ○ 3. Missing

2. Bowel sounds
   - ○ 1. Present
   - ○ 2. Absent
   - ○ 3. Decreased
   - ○ 4. Increased
   - ○ 5. Missing

3. Direct lower abdominal tenderness
   - ○ 1. None
   - ○ 2. Mild
   - ○ 3. Moderate
   - ○ 4. Severe
   - ○ 5. Missing

4. Lower abdominal rebound
   - ○ 1. None
   - ○ 2. Mild
   - ○ 3. Moderate
   - ○ 4. Severe
   - ○ 5. Missing

5. Costal-vertebral angle tenderness (CVAT)
   - ○ 1. None
   - ○ 2. R
   - ○ 3. L
   - ○ 4. Bilateral

Inguinal Nodes

6. Nodes tender
   - ○ 1. Yes
   - ○ 2. No
   - ○ 3. Missing

7. Nodes enlarged
   - ○ 1. Yes
   - ○ 2. No
   - ○ 3. Missing

External Genital Findings

8. Erythema
   - ○ 1. Yes
   - ○ 2. No
   - ○ 3. Missing

9. Edema
   - ○ 1. Yes
   - ○ 2. No
   - ○ 3. Missing

10. Vaginal discharge at introitis
    - ○ 1. Yes
    - ○ 2. No
    - ○ 3. Missing

11. Fissures
    - ○ 1. Yes
    - ○ 2. No
    - ○ 3. Missing

12. Excoriations
    - ○ 1. Yes
    - ○ 2. No
    - ○ 3. Missing

13. Vesicles
    - ○ 1. Yes
    - ○ 2. No
    - ○ 3. Missing

14. Pustules
    - ○ 1. Yes
    - ○ 2. No
    - ○ 3. Missing

15. Ulcers
    - ○ 1. Yes
    - ○ 2. No
    - ○ 3. Missing

16. Warts
    - ○ 1. Yes
    - ○ 2. No
    - ○ 3. Missing

17. Other external findings
    - ○ 1. Yes
    - ○ 2. No
    - ○ 3. Missing

18. Urethral erythema
    - ○ 1. Yes
    - ○ 2. No
    - ○ 3. Missing

19. Urethral edema
- ○ 1. Yes
- ○ 2. No
- ○ 3. Missing

20. Urethral exudate
- ○ 1. Yes
- ○ 2. No
- ○ 3. Missing

21. Bartholin's duct enlarged
- ○ 1. Yes
- ○ 2. No
- ○ 3. Missing

22. Bartholin's duct tender
- ○ 1. Yes
- ○ 2. No
- ○ 3. Missing

23. Bartholin's duct exudate
- ○ 1. Yes
- ○ 2. No
- ○ 3. Missing

Vagina

24. Erythema
- ○ 1. Yes
- ○ 2. No
- ○ 3. Missing

25. Warts
- ○ 1. Yes
- ○ 2. No
- ○ 3. Missing

26. Ulcers
- ○ 1. Yes
- ○ 2. No
- ○ 3. Missing

27. Other vaginal findings
- ○ 1. Yes
- ○ 2. No
- ○ 3. Missing

Characteristics of Vaginal Discharge

28. Is discharge assessable?
- ○ 1. Yes
- ○ 2. No
- ○ 3. Missing

29. Menstruating at this exam?
- ○ 1. Yes
- ○ 2. No
- ○ 3. Missing

30. Discharge Present
- ○ 1. Yes
- ○ 2. No
- ○ 3. Missing

31. Amount of discharge
- ○ 1. Minimal
- ○ 2. Moderate
- ○ 3. Profuse
- ○ 4. Missing

32. Discharge color
- ○ 1. white
- ○ 2. Clear
- ○ 3. Yellow
- ○ 4. Brown
- ○ 5. Bloody
- ○ 6. Missing

33. Discharge viscosity
- ○ 1. Thin
- ○ 2. Average
- ○ 3. Thick
- ○ 4. Missing

34. Discharge consistency
- ○ 1. Non-homogenous (normal)
- ○ 2. Homogenous
- ○ 3. Curdy/plaques
- ○ 4. Frothy
- ○ 5. Other
- ○ 6. Missing

35. Discharge distribution
- ○ 1. Pooled
- ○ 2. Diffuse
- ○ 3. Patches
- ○ 4. Missing

36. Discharge odor
- ○ 1. None
- ○ 2. Foul
- ○ 3. Fishy
- ○ 4. Missing

Cervical Characteristics

37. Ectopy
- ○ 1. None
- ○ 2. < 25%
- ○ 3. 25% - 49%
- ○ 4. 50% - 74%
- ○ 5. 75% - 100%
- ○ 6. Missing

38. Erythema
- ○ *1. None*
- ○ *2. Mild*
- ○ *3. Moderate*
- ○ *4. Severe*
- ○ *5. Missing*

39. Edema
- ○ 1. None
- ○ 2. Mild
- ○ 3. Moderate
- ○ 4. Severe
- ○ 5. Missing

40. Bleeds easily with contact
- ○ 1. None
- ○ 2. Mild
- ○ 3. Moderate
- ○ 4. Severe
- ○ 5. Missing

41. Ulcers
- ○ 1. Yes
- ○ 2. No
- ○ 3. Missing

42. Other cervical findings
- ○ 1. Yes
- ○ 2. No
- ○ 3. Missing

Cervical Mucus

43. Cervical mucus amount
- ○ 1. Minimal (to os)
- ○ 2. Moderate (on face)
- ○ 3. Profuse (pools)
- ○ 4. Missing

44. Color of cervical mucus
- ○ 1. Clear
- ○ 2. Opaque white
- ○ 3. Translucent white
- ○ 4. Yellow
- ○ 5. Brown
- ○ 6. Bloody
- ○ 7. Missing

45. Viscosity of cervical mucus
- ○ 1. Thin
- ○ 2. Average
- ○ 3. Thick
- ○ 4. Missing

Assessment of Uterus

46. Difficulty of assessment
- ○ 1. No uterus
- ○ 2. Assessable
- ○ 3. Difficult to assess
- ○ 4. Missing

47. Uterine position
- ○ 1. Anterior
- ○ 2. Posterior
- ○ 3. Midline
- ○ 4. Difficult to assess
- ○ 5. Missing

48. Uterine size
- ○ 1. Normal
- ○ 2. Enlarged
- ○ 3. Difficult to assess
- ○ 4. Missing

49. If enlarged, weeks size

[ ][ ]

50. Cervical motion tenderness
- ○ 1. None
- ○ 2. Mild
- ○ 3. Moderate
- ○ 4. Severe
- ○ 5. Missing

51. Uterine tenderness
- ○ 1. None
- ○ 2. Mild
- ○ 3. Moderate
- ○ 4. Severe
- ○ 5. Missing

Examination of Adnexae

52. Adnexal tenderness
- ○ 1. Yes
- ○ 2. No
- ○ 3. Difficult to assess
- ○ 4. Missing

53. Adnexal mass - Right
- ○ 1. Present
- ○ 2. Absent
- ○ 3. Missing

54. Adnexal mass - Left
- ○ 1. Present
- ○ 2. Absent
- ○ 3. Missing

55. Mass size - R - diameter (cm)

[ ][ ]

56. Mass size - L - diameter (cm)

[ ][ ]

57. Wet Prep

Trich	○ Yes	○ No	○ Missing
Hyphae	○ Yes	○ No	○ Missing
>= 20% Clue	○ Yes	○ No	○ Missing
Amine	○ Yes	○ No	○ Missing
WBC	○ Yes	○ No	○ Missing

pH [ ].[ ]

DOD Study
Physical Examination Form
Study Code _____

Litmus Card

58. pH
- ○ 1. Positive
- ○ 2. Negative
- ○ 3. Missing

59. Amine
- ○ 1. Positive
- ○ 2. Negative
- ○ 3. Missing

60. Yeast
- ○ 1. Positive
- ○ 2. Negative
- ○ 3. Missing

DOD Study Lab Results Form

dodlabp
(02-06-97)

Draft

Study code

Study ID

Specimen Date ___ / ___ / ___

Enrollment Site

0 0. Pitt 0 1. Health Dept. 0 2. Other

Other Site

Leukocyte Esterase

○ neg ○ moderate
○ trace ○ large
○ small ○ not done

Nitrite

○ 1.pos
○ 2.neg
○ 3.not done

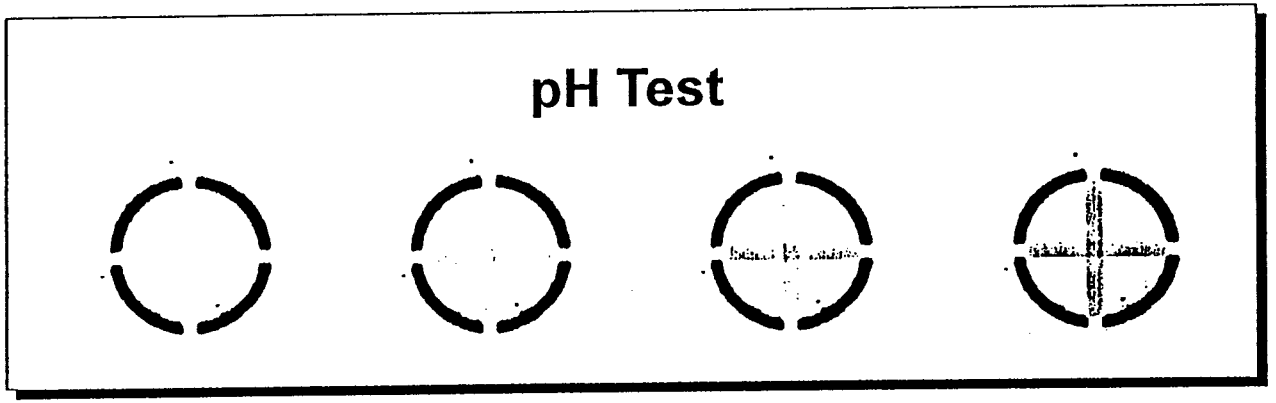
TEST	CONCENTRATION	RESULT
Lactoferrin Elisa	nanograms/ ml	○ not done
Defensins	nanograms/ ml	○ not done
Chlamydia PCR	absorbance reading	○ 1.pos ○ 2.neg ○ 3.not done
N.gonorrhoeae PCR	absorbance reading	○ 1.pos ○ 2.neg ○ 3.not done
Trichomonas PCR	absorbance reading	○ 1.pos ○ 2.neg ○ 3.not done

TEST	CULTURE	RESULT
Trichomonas	1 ○ 2 ○ 3 ○ 4 ○ 5 ○ Day Positive	○ 1.pos ○ 2.neg ○ 3.not done
Yeast	1+ ○ 2+ ○ 3+ ○ 4+ ○	○ 1.pos ○ 2.neg ○ 3.not done
N.gonorrhoeae	1+ ○ 2+ ○ 3+ ○ 4+ ○	○ 1.pos ○ 2.neg ○ 3.not done
Chlamydia		○ 1.pos ○ 2.neg ○ 3.CPE ○ 4.not done
Urine	quantity ____ code ___ quantity code 1 ___.___	urine result 1 ○ 1.pos ○ 2.neg ○ 3.not done
	quantity ____ code ___ quantity code 2 ___.___	urine result 2 ○ 1.pos ○ 2.neg ○ 3.not done

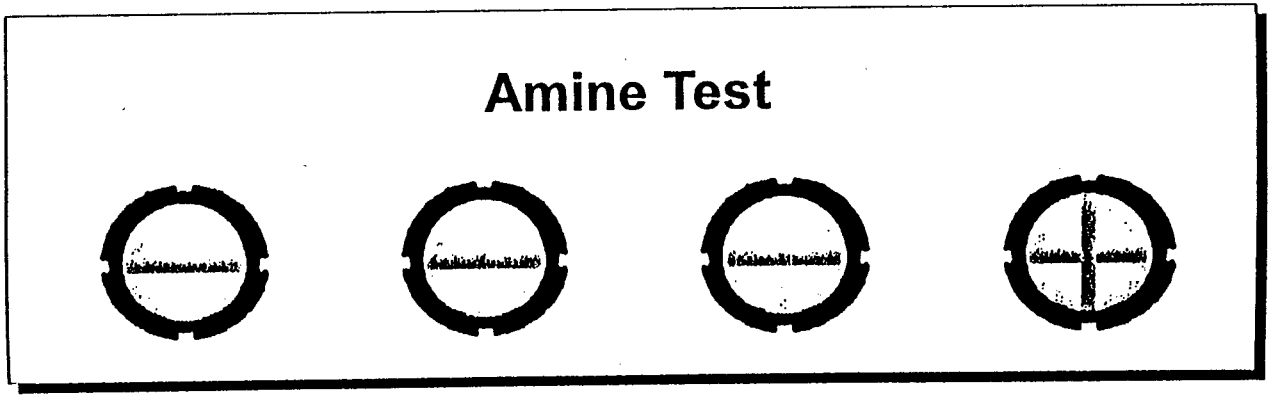
GRAM STAIN RESULTS	VAGINA
Lacto large positive rods	
Lacto intermediate positive rods	
G. vaginalis small variable rods	
Positive cocci	
Neg Rods: small	
Neg Rods: curved	
Neg Rods: fusiform	
Neg Rods: coliform	
Yeast	
Sperm	
Score	
Categorized Score	normal ○ intermediate ○ BV ○

VAG GBS	RECTAL GBS
neg ○	neg ○
broth ○	broth ○
1+ ○	1+ ○
2+ ○	2+ ○
3+ ○	3+ ○
4+ ○	4+ ○
not done ○	not done ○
___ - ___	___ - ___
Vag GBS StockLocation	Rectal GBS Stock Location

Examples of Test Results



Negative | Weak Positive | Intermediate Positive | Strong Positive



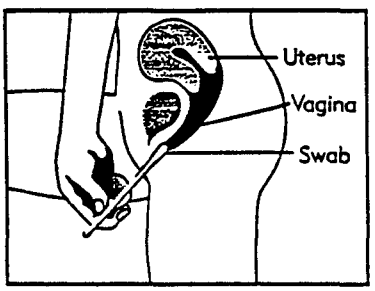
SAMPLE COLLECTION:

Step 1. Take a Vaginal Sample:

- Relax and take your time.
- Use a comfortable position for taking a vaginal sample.
- Recommended positions:
  - Squat with knees bent, feet apart
  - Stand with knees bent, one foot on toilet
  - Lie on your back with your knees bent

Step2. Insert the Swab:

- Remove the swab from the wrapper.
- Holding the middle of the swab, gently insert the swab into the vagina about 3" (as far as a tampon).
- Rotate several times swiping the sides of the vagina.



- Remove the swab from the vagina and place the swab tip in the tube.
- If you see any blood on the swab, do not continue the test. The red color will interfere with your result.

DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
504 SCOTT STREET
FORT DETRICK, MARYLAND 21702-5012

REPLY TO
ATTENTION OF:

MCMR-RMI-S (70-1y) 23 Aug 01

MEMORANDUM FOR Administrator, Defense Technical Information Center (DTIC-OCA), 8725 John J. Kingman Road, Fort Belvoir, VA 22060-6218

SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to the technical reports listed at enclosure. Request the limited distribution statement for these reports be changed to "Approved for public release; distribution unlimited." These reports should be released to the National Technical Information Service.

2. Point of contact for this request is Ms. Judy Pawlus at DSN 343-7322 or by e-mail at judy.pawlus@det.amedd.army.mil.

FOR THE COMMANDER:

Encl

PHYLIS M. RINEHART
Deputy Chief of Staff for
Information Management

Reports to be Downgraded to Unlimited Distribution

ADB241560	ADB253628	ADB249654	ADB263448
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